Agricultural and Forest Meteorology 265 (2019) 245–251

Contents lists available at ScienceDirect

Agricultural and Forest Meteorology

journal homepage: www.elsevier.com/locate/agrformet

Ectomycorrhizal fungi respiration quantification and drivers in three differently-aged larch plantations

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ARTICLE INFO

Keywords:
Ectomycorrhizal respiration
Rhizosphere respiration
Micro-pore meshes method
Stand age
Controlling factor

ABSTRACT

Soil respiration (Rs) is generally partitioned into autotrophic respiration (by roots and mycorrhizae) and heterotrophic respiration (by decomposers). Boreal and temperate forests are widely associated with ectomycorrhizal (EM) fungi, which play a critical role in belowground carbon dynamics. However, the magnitude and factors controlling EM fungal respiration (Rem) have not been well studied in field experiments. In this study, we quantified Rem using the micro-pore mesh method in three *Larix principis-rupprechtii* plantations of different ages (i.e., 11-, 20-, and 45-year-old, representing sapling, young, and mature stands, respectively) during the growing seasons from 2014 to 2016 in North China. The results showed clear seasonality of Rem, with an initial increase in spring (May and June), peak in summer (July and August), and decrease in autumn (September and October). Rem represented 37, 47 and 39% of rhizosphere respiration in the sapling, young, and mature stands, respectively, with an average of 41% across the three stands, indicating that a significant portion of rhizosphere respiration originated from EM fungi in larch plantations. Rem was positively correlated with soil temperature and the annual fine root increment, but negatively correlated with soil moisture when values exceeded 0.055 $cm^3\ cm^{-3}$. Stand age had no significant effects on Rem, but Rem and the contribution of Rem to Rs in summer in the sapling stand was significantly higher than values in the young and mature stands. These results may be due to the higher values of soil temperature and annual fine root increment and lower soil inorganic nitrogen in the sapling stand relative to the other two stands. Overall, our results emphasize the importance of partitioning and quantifying EM fungal respiration to improve our understanding and predictions of belowground carbon dynamics under global climate change.

1. Introduction

Carbon dioxide (CO_2) emission from soil (soil respiration, Rs) has attracted a great deal of interest during recent decades (Högberg et al., 2001; Lee et al., 2004; Piao et al., 2008; Raich and Schlesinger, 1992). Due to its huge magnitude, even small changes in Rs can have notable impacts on atmospheric CO_2 concentration, with potential feedback on the global climate (Peng et al., 2009; Piao et al., 2008, 2018; Raich and Schlesinger, 1992; Wang et al., 2014a). Rs is commonly partitioned into autotrophic respiration (Ra, or rhizosphere respiration, representing CO_2 released by roots, mycorrhizal fungi, and other rhizosphere organisms) and heterotrophic respiration (Rh, representing CO_2 released during the decomposition of dead organic matter by free-living microbes and fauna) (Heinemeyer et al., 2007). Numerous studies have primarily focused on how Rs and/or its two main components, Ra and Rh, respond to biotic and/or abiotic factors in different ecosystems (Hursh et al., 2017; Wang et al., 2014b; Zhong et al., 2016; Zhou et al., 2016), whereas less attention has been paid to the role of mycorrhizal fungi associated with roots (Heinemeyer et al., 2007).

The majority of plants in terrestrial ecosystems associate with mycorrhizal fungi (e.g., 94%, Brundrett, 2009). These host species rely on mycorrhizal fungal symbionts for nutrient uptake (Lambers et al., 2008; Leake et al., 2004) and in turn allocate carbon (C) to mycorrhizal fungi (Cairney, 2012; Hobbie, 2006). Boreal and temperate forest ecosystems are frequently associated with ectomycorrhizal (EM) fungi (Dickie et al., 2013; Gao and Yang, 2010; Read et al., 2004). EM fungi can produce extramatrical mycelium from EM root tips into soil to forage for nutrients (Anderson and Cairney, 2007); such mycelium can reach

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https://doi.org/10.1016/j.agrformet.2018.11.024
Received 29 August 2018; Received in revised form 21 November 2018; Accepted 21 November 2018
Available online 27 November 2018
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Table 1
Top soil (0–10 cm) and stand characteristics of the three studied larch plantation stands in 2010.

Stand age (years)	11 (sapling stand)	20 (young stand)	45 (mature stand)
Location (latitude, longitude)	42°23.3′ N, 117°14.0′ E	42°23.6′ N, 117°14.1′ E	42°23.9′ N, 117°14.8′ E
Stand density (tree ha^{-1})	2640 ± 157b	3160 ± 129a	870 ± 48c
Tree height (m)	2.5 ± 0.2c	7.8 ± 0.3b	15.8 ± 1.6a
DBH (cm)	2.4 ± 0.3c	7.6 ± 0.1b	19.9 ± 2.8a
Soil bulk density (g cm^{-3})	1.47	1.50	1.47
Soil texture (sand, silt, clay, %)	54.2, 28.3, 17.5	66.9, 20.0, 13.1	74.0, 15.4, 10.6
Soil organic carbon (Mg ha^{-1})	39.5 ± 1.5b	53.2 ± 5.1a	47.9 ± 3.6ab
Soil C:N	9.0 ± 0.2b	9.7 ± 0.2a	8.9 ± 0.3b
Soil NH_4^+ + NO_3^- (mg kg^{-1})	4.2 ± 0.9b	6.9 ± 0.6a	5.9 ± 1.3a
Soil pH	6.7 ± 0.2a	6.4 ± 0.2ab	6.3 ± 0.2b
Soil temperature (°C)	14.8 ± 0.1a	11.0 ± 0.4b	13.2 ± 0.3b
Soil moisture (cm^3 cm^{-3})	0.036 ± 0.001b	0.042 ± 0.000a	0.042 ± 0.001a
Annual litterfall biomass (g m^{-2} $year^{-1}$)	182.7 ± 14.4b	326.7 ± 27.6a	235.9 ± 21.9b
Annual fine roots increment (g m^{-3} $year^{-1}$)	1076.4 ± 348.1a	855.3 ± 138.2a	541.9 ± 122.5a

Note: Different letters within a row indicate significant differences among stands at $P < 0.05$. DBH is the diameter at breast height. Soil temperature, soil moisture, annual litterfall biomass, and annual fine root increment are the mean values during the growing season from 2014 to 2016. Values are means ± standard error.

up to 8000 m per meter length of root and can account for up to one-third of soil microbial biomass in coniferous forests (Heinemeyer et al., 2007; Högberg and Högberg, 2002; Leake et al., 2004). Heinemeyer et al. (2007) reported that EM fungal respiration (Rem) represented a larger contribution (25%) than root respiration (15%) to forest soil CO_2 efflux in a 15-year-old lodgepole pine (*Pinus contorta*) forest. Langley et al. (2006) showed that EM colonization slowed root decomposition in a piñon pine (*Pinus edulis*) woodland, i.e., the initial C mass lost was 7 and 19.3% in EM roots and non-mycorrhizal roots, respectively, after 2 years of field decomposition. Although EM fungi play a critical role in belowground C dynamics (Cairney, 2012; Wallander et al., 2010), the magnitude of Rem (which has often been simplistically included in Ra in previous studies) has been poorly quantified, and the contribution of Rem to soil CO_2 efflux has been largely ignored (Heinemeyer et al., 2007; Moyano et al., 2007).

Distinguishing the responses of Rem to different controlling factors would improve our understanding of soil C dynamics and allow for more accurate model predictions (Heinemeyer et al., 2007; Moyano et al., 2007, 2008). Soil temperature, soil moisture, and substrate supply (e.g., photosynthetic C and litterfall) are considered as the primary determinants of Rem (Heinemeyer et al., 2007; Moyano et al., 2008). For example, Heinemeyer et al. (2007) showed that Rem was dependent on substrate supply and responded strongly to soil moisture, but not soil temperature, in a young lodgepole pine forest in the United Kingdom. Additionally, stand age is another important forest characteristic that can influence the structure and function of ecosystems (Yao et al., 2018), e.g., net primary production (He et al., 2012) and the production of sporocarps by EM fungi (Bonet et al., 2004), thus potentially affecting CO_2 emissions through EM fungi. Nevertheless, few studies have assessed the controlling factors of Rem in plantation forests of different ages.

The area of plantation forests has increased rapidly during the past three decades, especially in temperate zones, e.g., China (Payn et al., 2015). Larch (*Larix* spp.) is an important coniferous tree species that is widely distributed in temperate and boreal zones (Gower and Richards, 1990; Mason and Zhu, 2014). For example, the area of larch plantations recently reached 2.61×10^6 ha in Northeast China (Chinese Ministry of Forestry, 2014). Therefore, soil CO_2 loss from this forest type likely has a key role in regulating regional C cycling and C budgets. Despite this, to our knowledge, no research has ever quantified Rem and its drivers in larch plantations of different ages. Therefore, in this study, we investigated Rem and its driving factors from 2014 to 2016 in three larch (*Larix principis-rupprechtii*) plantations of different ages in North China. The objectives were to (i) determine the magnitude and seasonal pattern of Rem among larch plantations of different stand ages, and (ii) identify the controlling factors of Rem. We hypothesized that Rem would contribute significantly to Ra and Rs, and would vary among different stands, given that our previous studies demonstrated that key environmental factors (e.g., soil temperature, soil moisture, and litterfall biomass) varied among the three stands (Ma et al., 2014; Yan et al., 2018a).

2. Materials and methods

2.1. Study site

The study was conducted at the Saihanba ecological station (42°24.723′N, 117°14.844′E, 1505 m a.s.l.) of Peking University, located in Saihanba National Forest Park, Hebei Province, China. The climate is semi-humid and characterized by long and cold winters and brief springs and summers. The mean annual temperature was –1.4 °C, minimum and maximum monthly mean temperatures of –21.8 and 16.2 °C in January and July, respectively, the mean annual precipitation was 450 mm, and the frost-free duration was 81 days based on long-term data from 1971 to 2010 (Ma et al., 2014). The topography is relatively flat, and the well-drained soils are predominantly sandy. Snowfall begins in mid-October, and snow melt occurs in early April. The depth of snow cover is generally no more than 30 cm in winter. The growing season lasts from May to October.

2.2. Experimental design

In August 2009, we selected three pure larch plantation stands aged 11, 20, and 45 years, which represented sapling, young and mature stands, respectively. Subsequently, all three stands (100 × 100 m) were fenced to minimize anthropogenic and herbivore disturbances. Three replicate plots (20 × 20 m each) were established in each stand, with a buffer zone of at least 10 m between plots (Ma et al., 2014). The distance between any two stands was less than 2 km, which avoided differences in climate conditions and soil type (Ma et al., 2014; Sun et al., 2014, 2016; Yan et al., 2018a). In addition, the three planted stands followed similar trajectories, as they were all in their first rotation and originated from stretches of primary forest that have been harvested during a large-scale industrial logging operation over the last century (Yan et al., 2018a). The basic characteristics of the three stands are outlined in Table 1.

2.3. Partitioning method

Three replicated groups of collars were inserted and randomly placed in each plot. Each group included four collar treatments (two collars with a mesh treatment and the other two with a no-mesh

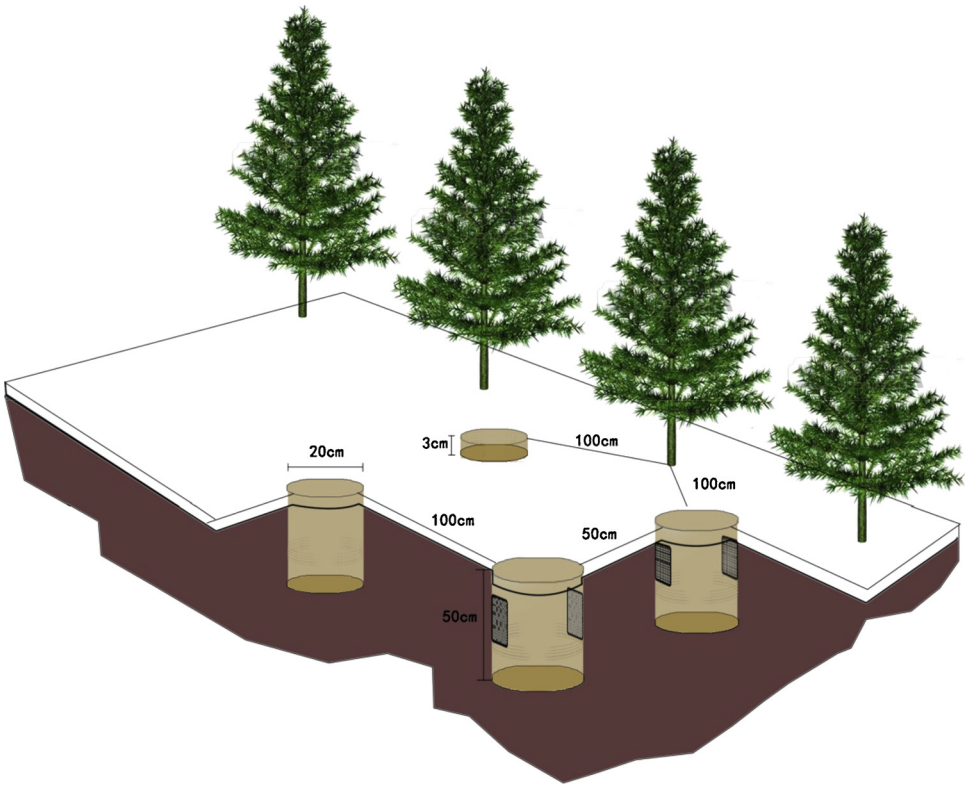

Fig. 1. Simplified schematic treatment design of the partitioning method. Briefly, a group of collars included four collars of treatments (depth of insertion and mesh type). The first collar (20 cm diameter and 50 cm height) had two 5 × 20-cm windows in its side, which were covered with 35-μm nylon mesh (Rm35); this mesh excluded roots but allowed in-growth of ectotrophic mycorrhizal (EM) hyphae. The second collar (20 cm diameter and 50 cm height) also had two 5 × 20-cm windows in its side, which were covered with 1-μm nylon mesh (Rm1) that excluded the in-growth of both roots and EM hyphae. The third (20 cm diameter and 50 cm height) and fourth (20 cm diameter and 11 cm height) collars had no mesh windows, and were used to measure total soil respiration (Rs) and heterotrophic respiration (Rh), respectively. The first, second and third collars were inserted 47 cm into the soil, while the fourth was inserted to 8 cm. The difference between Rm35 and Rm1 is the EM respiration (Rem), while the difference between Rs and Rh is the autotrophic or rhizosphere respiration (Ra).

treatment; Fig. 1) and different insertion depths (cm) into the soil. Briefly, both the first and second collars (20 cm diameter and 50 cm height) had two 5 × 20-cm windows cut into their sides that were covered with different-sized nylon mesh, one with 35-μm mesh (M35 treatment, the corresponding respiration was Rm35) that excluded roots but allowed the in-growth of ectotrophic mycorrhizal hyphae, and the other covered with 1-μm mesh (M1 treatment, the corresponding respiration was Rm1) that excluded roots and ectotrophic mycorrhizal hyphal in-growth. In April 2014, the first and second collars were both inserted into the soil to a depth of 47 cm to measure Rm35 and Rm1, respectively. Therefore, any difference between Rm35 and Rm1 was due to ectotrophic mycorrhizal respiration (i.e., Rem). This method followed Heinemeyer et al. (2007) and Moyano et al. (2007). Adjacent to the above-mentioned collars, a third collar (without mesh, 20 cm diameter and 50 cm height) was inserted into the soil to a depth of 47 cm in April 2010 and was used to investigate Rh. The fourth collar (without mesh, 20 cm diameter and 11 cm height) was inserted 8 cm into the soil in April 2010 to determine Rs. The depth of the litter layer can reach 5 cm, indicating that only the roots in the top 3 cm of soil would be injured by mechanical insertion of the collars, which was assumed to have no significant impact on Rs (Ma et al., 2014; Sun et al., 2014). Ra was determined by the difference between Rs and Rh. In total, 24 collars were inserted in each stand (2 groups of collars in each plot × 4 collars in each group × 3 plots in each stand). A simplified schematic treatment design of the partitioning method is illustrated in Fig. 1.

2.4. Respiration measurements

Measurements of respiration were conducted from May to October in 2014–2016, using a LI-8100 soil CO_2 flux system (LI-COR, Inc., Lincoln, NE, USA). Generally, Rs was measured twice a month, once early in the month and once late in the month. Specifically, nine measurements were made in 2014, with the first in late June and the last in late October. Ten measurements were conducted in both 2015 and 2016, with the first in late May and the last in early October. Along with the measurements of respiration, measurements of soil temperature and soil moisture near each collar were also automatically recorded to a depth 5 cm of topsoil using LI-COR 8100 temperature (8100-201) and moisture (8100-204) probes, respectively.

2.5. Annual litterfall and fine root biomass measurements

In May 2014, we randomly established two litter traps (1 × 1 m, with a mesh size of 0.5 mm) at a height of 0.8 m from the ground in each plot. Litterfall in the traps was collected monthly during the growing season, and then oven dried at 65 °C for at least 48 h to a constant weight. The final litterfall biomass was the average of the three plots in each stand from 2014 to 2016.

The annual fine root increment was measured using the in-growth method from 2014 to 2016. In May of each year, three soil root augers (10 cm diameter, with a mesh size of 1 cm) were randomly inserted into the soil to a depth of 40 cm in each plot (almost no roots are observed below depths of 40 cm; Ma et al., 2014). The augers were collected in October of each year, and roots were separated by sieving and washing. Finally, roots were weighed after being oven dried at 65 °C for more than 48 h to a constant weight. The final fine root biomass was the average of the three plots in each stand from 2014 to 2016.

2.6. Data analysis

The most common van't Hoff equation was used to assess the responses of Rs, Ra and Rem to soil temperature (Lloyd and Taylor, 1994; Tu et al., 2013) using the following equation:

$$R = \alpha e^{\beta(T-10)} \quad (1)$$

where R is the Rs, Ra or Rem flux (μmol m^{-2} s^{-1}); α and β are the fitted parameters; and T is soil temperature (°C) at a depth of 5 cm. The calculation of Q_{10} was as follows:

$$Q_{10} = e^{10\beta} \quad (2)$$

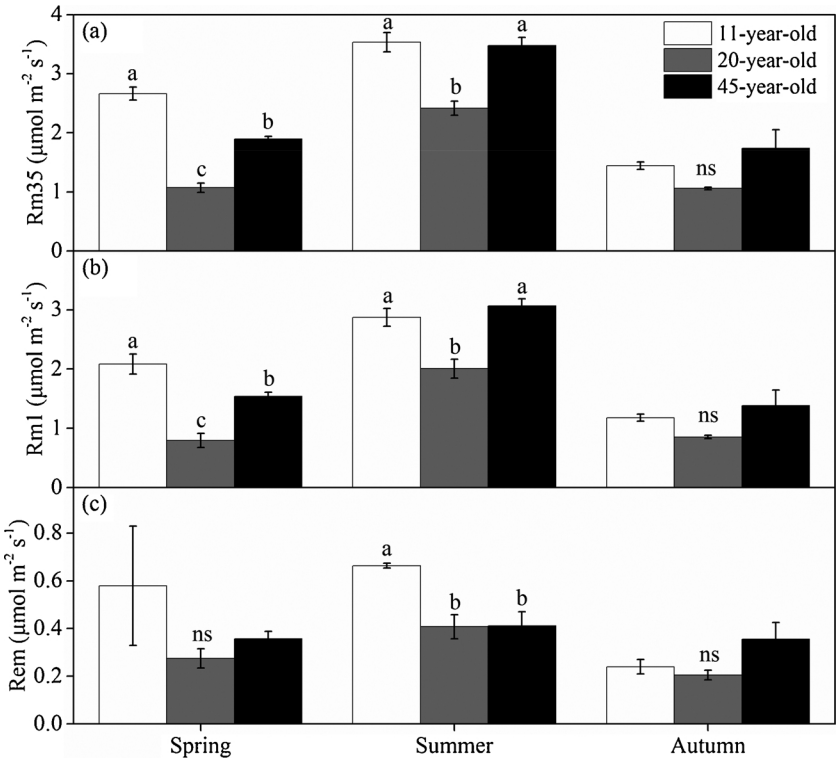

Fig. 2. (a) Rm35, (b) Rm1 (c) and ectomycorrhizal respiration (Rem) in spring (May and June), summer (July and August), and autumn (September and October) for the 11-, 20-, and 45-year-old larch plantations. Different letters indicate significant differences among the three stands at $P < 0.05$. Values are given as the means ± standard error (n = 3).

where Q_{10} is the temperature sensitivity, which describes the change in the Rs rate for each 10 °C increase in soil temperature.

Kolmogorov–Smirnov and Levene's tests were used to test the normality and homogeneity of variances, respectively. Data were calculated as the mean of the three plots in each stand from 2014 to 2016. One-way analysis of variance was used to test for differences among the three stands in Rm35, Rm1, Rem, Rem/Ra or Rem/Rs in each season. If the difference was significant, then *post hoc* multiple comparisons were conducted using the least significant difference test. Relationships between Rem and soil temperature, soil moisture, annual litterfall biomass, and annual fine root increment were analyzed using linear regression. All statistical analyses were conducted in SPSS ver. 13.0 for Windows (SPSS Inc., Chicago, IL, USA). The significance level was set at $P < 0.05$.

3. Results

3.1. Quantification of Rem

The Rm35, Rm1 and Rem in the three stands exhibited clear seasonal variations, peaking in summer and reaching minimums in autumn (Fig. 2). In summer, Rem significantly differed among the three stands; in contrast, values did not differ among stands in spring or autumn (Fig. 2).

Throughout the growing season, Rem ranged from 0.24 to 0.66, 0.20 to 0.41, and 0.35 to 0.41 μmol m^{-2} s^{-1} in the sapling, young, and mature stands, respectively (Fig. 2). The relative contributions of Rem to Ra (the difference between Rs and Rh) and total Rs were 37% and 14%, 47% and 14%, and 39% and 15% in the sapling, young, and mature stands, respectively (Fig. 3). However, the relative contribution of Rem to Ra did not significantly differ among the three stands, whereas the relative contribution of Rem to Rs differed significantly among stands in summer (Fig. 3).

The Q_{10} of Rem ranged from 3.40 in the mature stand to 4.86 and 5.69 in the young and sapling stands, respectively, with an average of 4.65 across the three stands (Table 2). The Q_{10} values of Rem were higher than the corresponding values for Rs and Ra (Table 2).

3.2. Relationships between Rem and driving factors

Across all three stands from 2014 to 2016, Rem was significantly positively correlated with soil temperature ($R^2 = 0.30$, $P = 0.003$) and annual fine root increment ($R^2 = 0.24$, $P = 0.009$), but significantly negatively correlated with soil moisture ($R^2 = 0.45$, $P = 0.048$), when soil moisture was greater than 0.055 cm^3 cm^{-3}. Rem was not significantly related to annual litterfall biomass ($R^2 = 0.047$, $P = 0.277$; Fig. 4).

4. Discussion

4.1. Quantification of Rem among the three larch stands

We observed clear seasonal variation in Rem in all three stands of larch, i.e., Rem values initially increased in spring, reached a maximum in summer, and decreased thereafter in autumn (Fig. 2). This seasonality in Rem was most likely due to fluctuations in the supply of aboveground photosynthates to the rhizosphere (Du and Fang, 2014; Högberg et al., 2001; Subke et al., 2011), as evidenced by previous studies of Ra in an oak–grass savanna (Tang et al., 2005), Rem in a pine forest (Heinemeyer et al., 2007) and arbuscular mycorrhizal fungal respiration in a barley field (Moyano et al., 2007). The importance of photosynthate supply to fluctuations in Rem are not surprising given that EM fungi are strongly dependent on carbohydrates from host plants; for instance, previous estimates indicate that up to 20% of net primary production flows to EM fungi (Hasselquist et al., 2010; Treseder and Allen, 2000).

Using the micro-pore mesh method (Heinemeyer et al., 2007; Moyano et al., 2007), our results demonstrated that Rem represented substantial proportions of Ra (average; 41%) and total Rs (average; 14%), which was consistent with our hypothesis and indicated that Rem could act as an important contributor to Rs. Our observed values of Rem were remarkably similar to those reported by Fahey et al. (2005), who estimated (using the indirect mass balance approach) that Rem represented 12% of soil CO_2 efflux in a northern hardwood forest ecosystem. Similarly, Hasselquist et al. (2010) documented that Rem

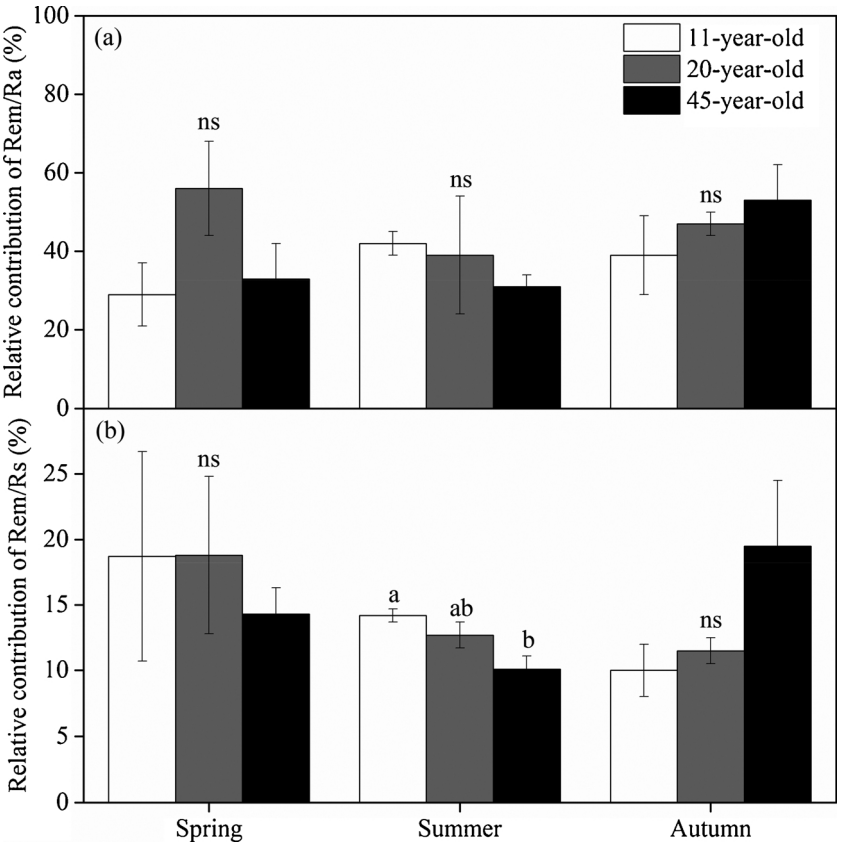

Fig. 3. Relative contributions of ectomycorrhizal respiration (Rem) to (a) autotrophic respiration (Ra) and (b) soil respiration (Rs) for the 11-, 20-, and 45-year-old larch plantations. Different letters indicate significant differences among the three stands at $P < 0.05$. Values are given as the means ± standard error (n = 3).

Table 2
Results of the exponential relationship between Rs, Ra and Rem with soil temperature at a 5-cm depth for the three stands (n = 36).

Component	Stand age (years)	Equation	Q_{10}	R^2
Rs	11-	$R = 1.80 \times 2.74^{(T-10)/10}$	2.74	0.71
	20-	$R = 1.84 \times 3.49^{(T-10)/10}$	3.49	0.83
	45-	$R = 2.07 \times 3.31^{(T-10)/10}$	3.31	0.71
Ra	11-	$R = 0.52 \times 2.99^{(T-10)/10}$	2.99	0.59
	20-	$R = 0.36 \times 2.75^{(T-10)/10}$	2.75	0.30
	45-	$R = 0.63 \times 2.89^{(T-10)/10}$	2.89	0.44
Rem	11-	$R = 0.13 \times 5.69^{(T-10)/10}$	5.69	0.44
	20-	$R = 0.20 \times 4.86^{(T-10)/10}$	4.86	0.39
	45-	$R = 0.20 \times 3.40^{(T-10)/10}$	3.40	0.22

accounted for 15% of soil CO_2 efflux in a conifer forest. Furthermore, the present estimation of the contribution of Rem to Ra (41%) was similar to the results of Subke et al. (2011), who observed a value of approximately 50% in a western hemlock (*Tsuga heterophylla*) stand using forest girdling, but higher than the value of 25% observed in a barley field using the micro-pore mesh method (Moyano et al., 2007). Thus, our results are consistent with the sparse field estimates of Rem by Heinemeyer et al. (2007), who demonstrated that Rem acted as a major component of Rs in a lodgepole pine forest.

Notably, the micro-pore mesh and trenching method may pose several limitations to the accuracy of Rem quantification. For example, when the collars are installed, roots inside the collar are killed, which could provide substrate for decomposers, thus may potentially lead to an overestimation of Rh. However, this may not the case in our study. Because the collars that used to measure Rh were inserted in May 2010 which was almost 4 years before the respiration measurements in our present study. In addition, soil moisture inside the collar may be altered due to the lack of uptake by roots. Finally, some saprotrophic fungal ingrowth may occur through the mesh. These limitations may potentially impact soil C emission. Nevertheless, our findings highlight the importance of Rem in forest soil C dynamics. Moreover, our results strengthen the concept that the previous strategy of partitioning of Rs into Ra and Rh should be modified (Moyano et al., 2007). Indeed, mycorrhizal fungal respiration should be included in these estimates, as Rem can represent a substantial proportion of soil CO_2 efflux in forest ecosystems, e.g., larch plantations.

In partial agreement with our hypothesis, stand age did not significantly affect Rem; however, Rem values and the relative contribution to Rs in the sapling stand in summer were significantly higher than values in the young and mature stands (Figs. 2 and 3). These results may be due to the higher soil temperatures (higher light penetration to the soil surface) observed in the sapling stand compared to the young and mature stands (Table 1). Because Rem was strongly dependent on soil temperature (Table 2), the higher soil temperatures in the sapling stand could lead to higher respiration flux as well as increased EM mycelial production by EM fungi (Clemmensen et al., 2006). In addition, the annual fine root increment in the sapling stand was higher, albeit not significantly, than the values in the young and mature stands (Table 1), which may allow for higher rate of EM fungal colonization and ultimately stimulate EM respiration (Hasselquist et al., 2010; Majdi et al., 2008; Wallander et al., 2001). Furthermore, previous studies have demonstrated that fungal biomass and EM mycelium production decrease with increasing soil nitrogen availability in forest ecosystems (Nilsson et al., 2005). In our study, soil inorganic nitrogen in the sapling stand was remarkably lower than values in the young and mature stands (Table 1), indicating that the production of EM mycelium and fungal biomass may have been higher in the sapling stand, which in turn could potentially promote CO_2 emission by EM fungi.

The average Q_{10} of Rem was 4.65 across the three stands (Table 2), which was higher than the Q_{10} of Ra (2.88). This result was likely due

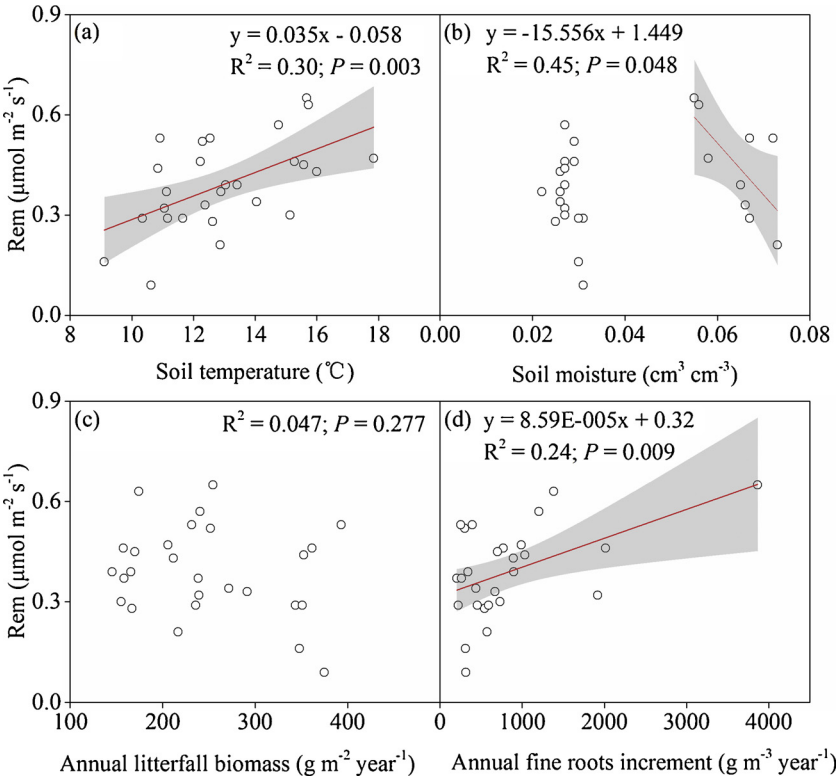

Fig. 4. Relationships between ectomycorrhizal respiration (Rem) and (a) soil temperature, (b) soil moisture, (c) annual litterfall biomass, and (d) annual fine root increment across the three stands from 2014 to 2016. Each point represents the mean value of the variables in each plot in each year (n = 27, 9 plots × 3 years). Linear regression (red line) and 95% confidence intervals (shaded gray area) are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to the fact that, compared to plant roots, EM fungi are more dependent on substrate supply (strongly related to temperature) (Heinemeyer et al., 2007; Söderström and Read, 1987). Thus, it would be questionable to estimate Q_{10} using Ra without considering the differences between EM fungi and roots, as has been the approach of most previous studies.

4.2. Environmental controls over Rem

Rs is regulated by both biotic (e.g., root density and photosynthesis) and abiotic (e.g., soil temperature and soil moisture) factors (Heinemeyer et al., 2006; Ryan and Law, 2005; Vargas and Allen, 2008). We observed that Rem was positively correlated with soil temperature (Fig. 4), indicating that this factor is one of the dominant drivers for respiration flux, as suggested by previous studies (Hursh et al., 2017; Ma et al., 2014; Wang et al., 2014c). The positive correlation between Rem and soil temperature in the present study contrasted with the findings of Heinemeyer et al. (2007), who observed a lack of response of Rem to soil temperature in a 15-year-old lodgepole pine forest in the United Kingdom. This discrepancy may be related to different fluctuations in substrate supply from photosynthesis to rhizosphere in different ecosystems (Heinemeyer et al., 2007; Lipp and Andersen, 2003). In addition, we found that Rem was negatively correlated with soil moisture when the latter exceeded 0.055 $cm^3\ cm^{-3}$; however, no response of Rem was observed when soil moisture was below 0.055 $cm^3\ cm^{-3}$ (Fig. 4). High soil moisture may negatively affect Rem, because low oxygen diffusion under saturated conditions limits EM fungal activity, in turn causing low Rem. In contrast, soil moisture did not exert a significant influence on respiration activity when moisture levels were moderate and not extreme (Moyano et al., 2007). Unfortunately, no soil moisture data between 0.031 and 0.055 $cm^3\ cm^{-3}$ were available in the present study, limiting our ability to more accurately explore the threshold of the Rem to soil moisture. These results were, to some extent, consistent with Heinemeyer et al. (2007), who also observed a high sensitivity of Rem to soil moisture.

In addition to abiotic factors, Rem was regulated by a biotic factor, i.e., the annual fine root increment positively affected Rem (Fig. 4). As discussed above, the most likely explanation is that higher fine root biomass may lead to more EM fungal colonization, and subsequently, increase in EM fungal activity and respiration (Hasselquist et al., 2010; Majdi et al., 2008; Wallander et al., 2001). Previous studies have demonstrated that high Rs rates are associated with greater lengths of fine roots (Vargas and Allen, 2008). In addition, the dependence of respiration on litter input as a substrate source was evident in a barley field (Moyano et al., 2007). However, we did not observe a significant relationship between Rem and annual litterfall biomass (Fig. 4). This lack of response was probably due to the observation that Rh was more dependent on litter input, while Rem relied more on photosynthates from leaves to roots and did not respond directly to changes in litterfall biomass.

Notably, the chronosequence approach, such as the one used here for larch stands of different ages, is controversial (Walker et al., 2010). However, the method remains necessary to study the temporal dynamics of ecosystem structure and function in forest ecosystems (Walker et al., 2010; Yan et al., 2018b). The three larch stands of different ages in the present study were similar in climate condition, soil type, and trajectory (Ma et al., 2014; Yan et al., 2018a); therefore, they were appropriate for investigating Rem and its drivers throughout stand development. Even so, further research is needed to confirm whether the present findings are representative of other plantation forests.

5. Conclusions

We quantified Rem and its drivers in three differently-aged larch plantations in North China. Unexpectedly, no significant differences in Rem were observed among the sapling, young, and mature stands. The respiration of EM hyphae can be an important contributor to soil CO_2 emission in larch plantations, as illustrated by the average relative contributions of 41% and 14% to Ra and total Rs, respectively. Rem was strongly influenced by abiotic factors (soil temperature and soil moisture) and a biotic factor (annual fine roots increment). The quantification of Rem and its driving mechanisms is necessary to accurately

assess and model C budgets in larch plantations.

Acknowledgements

We thank Dr. Shilong Piao for his critical comments and discussion for improving earlier versions of this manuscript. This work was supported by the National Natural Science Foundation of China (41801056; 31621091).

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